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Vol. 3

YELLOW FEVER COMMISSION

(WEST AFRICA).

THIRD REPORT

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PUBLISHED BY
J. & A. CHURCHILL, 7, GREAT MARLBOROUGH STREET, LONDON, W.

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THIRD REPORT

OF

THE YELLOW FEVER COMMISSION

(WEST AFRICA.)

In the Second Report of the Commission a brief account was given of the incidence of the disease in the West African Colonies up to May 31st, 1914.

2. At that date it was thought that the epidemic in Lagos had not completely died out; but since February 15th, 1914, no fresh case has been reported.

3. Cases have been notified since the date of the last report at the following places :—Jebba, Burutu and Onitsha, in Nigeria; Quittah, Axim, Somanya and Accra (Gold Coast Colony); and Tamale (Northern Territories, Gold Coast); the last to be noted being at Burutu, on the 23rd of September. The total number of cases up to the 30th of September, 1915, is therefore eighty-two. Of these forty-five have occurred in Europeans, including four Syrians, and thirty-seven in natives.

Twenty-five cases amongst Europeans and four amongst natives have proved fatal.

4. Owing to the exigencies of the public service it became necessary to recall to their ordinary duties those members of the West African Medical Staff who were engaged upon the work of

the Commission, and the outbreak of war has still further restricted the amount of attention which they and others in West Africa have been able to give to research.

5. It had been the intention of the Commission to establish a centre of work at Sierra Leone, and negotiations were in progress with men experienced in research in order to carry this into effect, but, for the reasons just stated, it has proved necessary to postpone the completion of this plan, although by the despatch of Mr. A. W. Bacot to Sierra Leone to study the life history of the *Stegomyia* mosquito, work for which he possesses special qualifications, the scheme has been in part fulfilled.

6. Since the issue of the last Report the Commission have had the advantage of the assistance of Dr. Andrew Balfour, C.M.G., who has been appointed by the Secretary of State to be a member of the Commission.

7. Two volumes containing reports from Investigators appointed by the Commission, and also other papers on subjects connected with the enquiry, have been published separately, under the title of "Yellow Fever Commission (West Africa), Reports on questions connected with the Investigation of Non-Malarial Fevers in West Africa."

It is believed that the records of these researches constitute a valuable addition to the literature of Yellow Fever. A third volume of Reports is in preparation.

8. In accordance with the intention expressed in their Second Report, the Commission have dealt in this Report with the questions of the occurrence and the significance of the bodies with which the name of Dr. Harald Seidelin is associated.

Its issue has been delayed, chiefly by difficulties connected with the production of the illustrations in the volumes of Investigators' Reports. Their prior publication was necessary, as in this Report there are many references to papers contained therein.*

---

* The references to these volumes in this Report are in the following form:—"I.R. Vol.——, p.——."

The Yellow Fever Bureau Bulletin (Liverpool) is referred to as "Y.F.B."

INTRODUCTORY.

In 1909 Dr. Seidelin published* an account of a supposed parasite which he had found in the blood of Yellow Fever patients, and two years later, in a paper entitled "Protozoon-like Bodies in the Blood and Organs of Yellow Fever patients,"† he gave a further and more complete description of these bodies, illustrated by plates showing in fifty separate figures the appearances which they presented when obtained from patients at periods varying from the third to the eighth day of the disease, and in one case on the fourteenth day. The majority of these forms were found in the blood, but some in organs, *e.g.*, the kidney, liver and spleen.

It is natural and necessary that any claim to have identified the virus of such a disease as Yellow Fever should receive the most rigid scrutiny, as, apart from the advance in medical science which such a discovery would mark, there are administrative problems of the highest importance which only await for their solution a knowledge of some certain method of identifying that disease, especially in its less severe forms.

The Commission now propose to review the evidence which has been adduced in favour of this claim and the arguments which have been brought forward in opposition to the view that these bodies are the parasite which gives rise to Yellow Fever.

Certain investigations which the Commission have undertaken, and researches on somewhat similar lines by other observers, have lately thrown fresh light upon the questions at issue, and would appear to justify the conclusions which are stated at the end of this Report.

It is of some importance that the discussion which has now been carried on for nearly five years should, if possible, be brought to an end, as its continuance has a tendency to divert research from other, and it may be more important, problems in connection with Yellow Fever.

It appeared to be desirable to allow Dr. Seidelin to state his case in his own words in order to avoid the possibility of controversy

---

* Berl. Klin. Wochenschr., 1909, No. 18.
† Journal of Path. and Bact. 15, 1911, p. 283.

arising from the omission of points which he might consider of importance in the evidence upon which he bases his claim, and this course has been adopted wherever it has been consistent with the necessary limits of this Report.

Forms of the Parasite in Human Blood.

In the later paper* to which reference has been already made the following statement occurs with regard to the various forms of the parasite and their relation to the day of the disease on which their presence was observed: —

> "During the earlier days in which the initial fever occurs I have found only extremely small forms with a minute chromatin point and a generally feebly stained blue protoplasm; the chromatin is not very red, but rather dark violet. A little later, during the reduction of the temperature, when this occurs on the third or fourth day, or in the period of reactive fever, there are still seen the same small forms; these are usually few in number, but there are in addition others, a little larger, which, whilst they consist chiefly of a rather deeply stained blue protoplasm, contain a still very small, but more purple-stained chromatin point.
>
> "Still later, in the last days of the fever, and, as it seems, far into the convalescent stage, we found in the corpuscles chromatin granules, these being well stained single or double, round or pear-shaped, and with a very small border of protoplasm or quite free of protoplasm.
>
> "All these forms are intraglobular. Outside the red corpuscles very few were observed * * * * * * * The observation of such small corpuscles is still more difficult outside than inside the erythrocytes. * * * * * * I consider their nature entirely doubtful; * * * * * * and am disposed to look upon them as artefacts.
>
> "I limit myself therefore to the reproductions of the larger extra-globular objects of whose preformed and probably protozoic nature there can be little doubt. There may, of course, be some doubt as to their specific nature, as they have as yet been found in very few cases. The two largest forms (Plate XXIX., Figs. 37 and 39), which have been found in two different cases, both fatal, and which show a quite characteristic structure and staining, recall somewhat the gametocytes of the malarial parasites, whilst the third shows a slight resemblance to some stages of the evolution of the Leishman-Donovan bodies. Moreover, there have been found in the same cases, and in a few others, similar but less fully developed forms. (pp. 283 and 284.)

* Journal of Path. and Bact. 15, 1911.

P. flavigenum *in the Tissues.*

" The positive findings in the tissues have been obtained principally in the kidneys. They have mostly been localised as a small number of groups in a section, not equally distributed over the whole. Two, or it may be three, different kinds of corpuscles have been observed.

" Firstly, the most characteristic present themselves as small, oval, faintly, or rather deeply bluish or somewhat reddish stained bodies with sharply defined, very small dark violet spots, generally one, sometimes two or, very seldom, three in each body. They are found in what seems to be but slightly altered or sometimes almost destroyed erythrocytes in capillaries or in renal tubules with degenerated epithelium.

" Their dimensions are from one-fifth to one-half of those of a normal red blood corpuscle. These bodies are so sharply limited and, on the whole, so well defined that I consider there can be little doubt as to their parasitic nature.

" Secondly, there were observed also in the renal capillaries very small elements which resemble division forms, being disposed in circles between, but apparently not inside, the blood corpuscles ; their number varies from five to nine ; they show a uniform violet colour, apparently without chromatin-point, but that may depend upon the technique employed (the sections coming from sublimate with acetic acid and being stained by Giemsa's method, while the other forms described were seen in sublimate-alcohol specimens stained by the Leishman method).

" Thirdly, finally, great numbers of a third class of small forms were found within large cells, probably endothelial, in liver and kidney sections ; they show a darker spot in their bodies, but do not show such clear-cut outlines or characteristic staining as those of the first class.

" This part of the work, the examination of the parasite in the tissues, is rather incomplete. * * * * * The very irregular, limited distribution in the kidney would suggest that this organ is not the principal site of multiplication of the microbe, and the same seems to be true only in a greater degree in the cases of the liver, pancreas, heart muscle, and spleen, which last organ in yellow fever does not, as a rule, show definite alterations, either macroscopically or microscopically. In freshly made preparations from spleen and bone marrow I have seen some microbes, and further study of the latter is evidently suggested." (p. 284.)

The Significance of the Discovery.

As to the significance of these observations, Dr. Seidelin states : —

" The possibility of artefacts I have discussed in my former article, and believe it can be excluded. Neither do I consider that there can be any serious doubt that we have to do with really foreign

elements and not with histological constituents, normal or abnormal, of the blood itself. Punctated erythrocytes are quite different from what I have described, and I have never seen any degeneration of these corpuscles really resembling the elements described.

"Blood platelets, though rather heteromorphous bodies, would seem never to become differentiated to a degree at all approximating the shape of the extraglobular forms I have observed.

"And I firmly believe that we can exclude this possibility with still greater confidence when we take into consideration the new facts now recorded regarding the findings in the sections of the organs. No kidney elements that I know of, and certainly no bacteria, would ever present the characteristics described. So I believe that I have demonstrated the presence in the blood and in some organs of micro-organisms which probably have not been seen or so interpreted before." (p. 285.)

Protozoal Nature of the Parasite.

"It would evidently be premature to pretend to determine the precise zoological place of the microbe. There can be little doubt as to its protozoic nature, considering its shape and staining reactions, but the forms observed do not suffice to enable us to make an exact diagnosis. The want of pigment and some of the morphological details, such as the double forms and the chromatin bodies almost without protoplasm, would suggest an affinity to the genus *Babesia* (*Piroplasma*),* whilst the aspect of the parasites in the kidney, and it may be the one apparently flagellated form observed, would seem to indicate a relation to the Leishman-Donovan bodies (*Herpetomonas*); in other details there is some resemblance to the malarial parasites." (p. 287.)

This organism was named by Dr. Seidelin *Paraplasma flavigenum.*

Other Forms of the Parasite in Inoculated Animals.

In a "Summary Report of Investigations carried out under the Yellow Fever Commission (West Africa)," † by Dr. Seidelin, reference is made to other forms of the parasite found in guinea pigs, which had been inoculated with blood from Yellow Fever patients at the Yaba Institute, Lagos, as follows:—

"In addition to the ordinary forms of the parasite, we found, in lungs and other organs, 'blue bodies'; we suggest that these bodies represent stages of division."

---

* *Footnote by Dr. Seidelin.* In case of a disease in imported cattle, very much like *piroplasmosis*, I found very numerous naked chromatin bodies.

† I.R. Vol. II, p. 423.

In a paper by Dr. Seidelin and Dr. Andrew Connal on "Experimental Yellow Fever in Laboratory Animals,"* further reference is made to the "blue bodies" as follows:—

"More interesting results were obtained from the examination of slide films (touch-preparations) from the organs.

"In these films ordinary forms of the parasite were found, occasionally in somewhat larger numbers than they had been found in the peripheral blood, or in the heart blood, but in no case could they be described as very numerous, and often they were quite scanty. Entire absence of the parasites in their ordinary forms was often observed when death took place a considerable time after the inoculation.

"Intracorpuscular division-forms, similar to those described in the peripheral blood, were sometimes met with, but were always rare. No other forms of special interest were noticed, except those which apparently represented the transition to the bodies now to be described." (p. 466.)

The "Blue Bodies."

"The most striking elements observed in the organ-touches were extracorpuscular bodies, which we will call provisionally and for the purposes of description 'blue bodies,' using the term commonly applied to the elements first described as 'Plasmakugeln' by Koch (1906) and others in East Coast Fever, in cattle. These bodies were in the first instance observed in 'lung-touches' from a guinea-pig which had been killed on the seventh day after inoculation. Afterwards they were seen in numerous cases in touch preparations from the various organs examined, i.e., lung, spleen, bone-marrow and liver, and though as a rule, most numerous either in lung or spleen touches, they were very rarely numerous in both these organs in the same animal.

"The advanced stages of the evolution of the blue bodies were found chiefly in the lungs. (pp. 466 and 467.)

Evolution of the Blue Bodies.

"After considerable experience had been gained in the examination of these elements it appeared evident that they developed from intracorpuscular forms, and their probable evolution can be described as follows:—

"The first stage seen differs but little from the ordinary intracorpuscular forms of *P. flavigenum*, though perhaps the chromatin is somewhat larger and the protoplasm more compact. These differences become more and more accentuated, and the parasite, whilst still intracorpuscular, grows to a size far in excess of that of the ordinary forms.

---

* I.R. Vol. II, pp. 466 sqq.

"It now appears that the parasite develops in one or other of two different ways. In some cases they apparently become extracorpuscular, and the chromatin is still compact, forming one comparatively large mass. In other cases the chromatin becomes arranged along the periphery of the blue body whilst the latter still remains intracorpuscular. In this position the chromatin appears less compact and stains less deeply than in the ordinary forms.

"The largest of these intracorpuscular bodies have a diameter of about one-fourth to one-third of that of an erythrocyte. Once having become free, whether at an earlier or a later stage, the parasites further increase in size and the chromatin divides. It appears that the chromatin, if already peripheral, retains this position and breaks up into a considerable number of small elements; how many we cannot state at present. On the other hand, in the forms with compact chromatin at one extremity, the body becomes rounded, and the chromatin becomes more or less central before it proceeds to division.

"In these cases the division appears to be a simple repeated fission; thus in some forms two nuclei are seen, in others four, and in others, again, finally eight. Immediately after, or even at the time of the last nuclear fission, the protoplasm evidently divides, as no forms have been observed in which eight chromatin particles were present in an undivided protoplasm. Thus, eight elements result from the division, and they seem to belong to two different types. In some of the round bodies elongated elements appear in which no further process of division has been observed. In other cases the resulting elements are more or less polygonal in shape, and in these it appears that further division takes place, and thus perhaps a similar result is obtained to that observed in the bodies with peripheral chromatin. It is also possible that a similar process occurs in the case of the elongated 'spores,' but transitional stages have not been observed. This possibility, according to which secondary divisions would take place in all cases, suggests itself, because the larger division-elements have never been seen in a free state. If this be so, it is probable that the division goes on until exceedingly minute elements, apparently consisting entirely of chromatin, have been formed. Such minute elements have been seen inside round 'capsules,' and they are the only elements of the series which have also been observed in a free state." (pp. 467 and 468.)

The Forms of the Parasite in Inoculated Guinea-Pigs.

In the same paper, in the "Discussion of results obtained,"* the following further reference is made to the forms of the parasite discovered in the inoculated guinea pigs and to the nature of the "blue bodies."

"The forms of the parasite observed in the peripheral blood were identical with those found in the blood of yellow fever patients.

* pp. 472 seq.

" They were, as a rule, very scarce, thus corresponding in this particular also to the conditions obtaining in human infections. No process of division could be demonstrated directly in fresh specimens, or by combination of forms found in dried films, but a certain number of individual forms were seen to possess from two to four chromatin granules. This, considered with the corresponding forms described by one of us (Seidelin, 1912, Pl. I, fig. 21) in human cases, suggests that occasionally a partial or complete division takes place in the peripheral blood. So far, no new facts had been observed with regard to the parasites. We learned no more from the examinations of the peripheral blood in our guinea-pigs than we had known beforehand." (p. 472.)

The Blue Bodies in Inoculated Guinea-pigs.

" The examination of organ-touches proved more successful. The forms which have been described above were strikingly different from those observed in the peripheral circulation. So different were they, in fact, that the question arose whether they were of an entirely different nature. Their parasitic origin could not be doubted, their morphological characters pointing so distinctly to a protozoic organism; but the possibility had to be considered of their representing other spontaneously-occurring parasites. We had to consider this question very seriously, because the parasites show a certain resemblance to the elements first observed by Chagas (1909) in guinea-pigs' lungs, and regarded by him as a stage in the evolution of *Trypanosoma cruzi*, whilst it has later been declared by others (see Aragao, 1913) to be a lung-parasite of guinea-pigs with no relationship whatsoever to the trypanosome." (pp. 472 and 473.)

Nature of the Blue Bodies.

" Our elements are evidently not identical with those described by Chagas, but there is the possibility that they might belong to the same group. We believe, however, that we can discard this hypothesis.* Two important factors are against it. One reason is that we have, as far as we can judge by the morphological characters, found transitional stages from small intracorpuscular elements to the fully developed dividing forms, the intermediate stages being intracorpuscular bodies larger than the initial ones, and free bodies smaller than the fully developed elements. These initial bodies show great resemblance to ordinary forms of *P. flavigenum*, and if this interpretation of our observations be correct, then we have to do with a blood parasite, and not with one of the lung. The correctness of this view is confirmed by the frequent finding of similar though not fully developed forms in other organs, especially in the spleen, and

Footnote by Dr. Seidelin.—The hypothesis of a mixed infection may, however, be admitted. The 'sporulating bodies' might differ essentially from the 'blue bodies'; in the case of the latter we believe that we can safely exclude the possibility of their being lung-parasites, whilst in the case of the former the question must at present be left undecided. For further discussion of this point see also Seidelin: Division forms of parasites in the organs of guinea-pigs infected with *Paraplasma flavigenum* (*Journ. Pathol. & Bacteriol.*, Cambridge, Jan., 1915, XIX, 3, pp. 338—340); and Connal and Johnston, Natural infection of guinea-pigs (*infra*, pp. 595 sqq.).

by the occasional finding of advanced stages of division in various organs.

"Secondly, we never found the same parasite in non-infected guinea-pigs, although a considerable number of these died and were subjected to a careful post-mortem examination, as above stated." (p. 473.)

The Relation of the Blue Bodies to Paraplasma flavigenum.

"The only objection, we believe, which can be raised against our view of regarding these division forms as a link in the life-cycle of *P. flavigenum* is that they have been found neither in human organs nor in the organs of other animals experimented upon. Further researches will have to be carried out in this connection, in view of our observations on guinea-pigs, but at the present time it appears that the objection is of no great importance. A comparison with the plates in several of Seidelin's earlier publications will show that, as a matter of fact, elements closely resembling the intracorpuscular and some of the extracorpuscular forms of blue bodies have already been figured without their true importance having been realised. The failure to find the more advanced stages in the human host may be explained in different ways. These stages are comparatively scarce, and they seem to occur only at certain periods; besides, they appear to change soon after death, as we have found them only in those cases in which the post-mortem has been made immediately or shortly after the death of the animal. Any of these circumstances would easily account for the absence of the division forms in earlier observations.

"It appears, as far as our data go, that the formation of 'blue bodies' may start in various organs of the body, but that their complete evolution can only take place in the lungs. Possibly further research will show that it may occur also in the spleen. Their occasional appearance in the peripheral blood may depend either on their exceptional formation there, or upon their being casually washed out from the internal organs by the blood current." (pp. 473 and 474.)

Morphology of the Blue Bodies.

"The morphological appearances of the 'blue bodies' suggests that they are sexual forms, gametocytes. One would, however, *a priori* feel inclined to adopt the view that in a mosquito-transmitted disease the sexual evolution of the parasite would take place in the insect-host, especially as there is some reason to believe that under ordinary conditions the transmission takes place only after a certain period of evolution of the parasite in the mosquito. On the other hand, this preconceived idea may be erroneous, and it may here be mentioned that Finlay (1903) suggested many years ago that the conditions in yellow fever are probably the reverse of those obtaining in malaria, *i.e.*, that in yellow fever the sexual stage of the parasite would be found in the human host and the asexual stage in the mosquito.

"Adopting the first view, the morphological appearances of the 'blue bodies' would correspond quite well to the theory of a double schizogonic process, the first stage being the formation of eight elements secondarily subdividing, probably inside the same 'cyst-membrane,' into a very large number of exceedingly minute merozoites.

"This discussion is at the present moment entirely theoretical, but it would appear that the following hypothesis possesses a certain probability. The parasites are present, to begin with, in the blood-stream as more or less irregular 'ring,' or 'pear,' or 'rod' shaped forms, and they divide, in the blood corpuscles, into four or five merozoites which infect other red-cells. Some of these newly-formed elements are compact, and develop no clear space in their interior. They retire into the internal organs and grow to a relatively excessive size; earlier or later they become free and divide into eight elements. These elements remain inside the original membrane of the 'blue body,' and undergo a second division, the result being the formation of minute elements which become free and presumably pass into the blood current where they would have a chance of being taken up by mosquitoes.

"Several additions must be made to this hypothesis. The 'blue bodies' appear to leave the erythrocytes at very different stages of their development, some becoming free whilst still of a small size, others not doing so until they appear about fully developed. In some cases it would appear that even the division may take place inside the remains of an erythrocyte, as several fully developed forms have been seen in which the 'membrane' shows a staining very similar to that of hæmoglobin, thus giving the impression of representing the remains of almost completely digested erythrocytes. The 'blue bodies' may become phagocytosed by mononuclear leucocytes at any stage of their evolution. Whether they can continue their development in the leucocytes we have no means of discussing." (pp. 474 and 475.)

Two Groups of Blue Bodies.

"The 'blue bodies' would appear to belong to two groups, those with a pale protoplasm and fairly abundant chromatin, which as a rule occupies a peripheral position, and those with a more deeply blue-staining protoplasm and a scanty loose centrally-placed chromatin mass. This, as mentioned above, raises the question whether a sexual process takes place in the human host." (p. 475.)

Evolution of the Parasite in the Mosquito.

In the "Summary Report of Investigations carried out under the Yellow Fever (West Africa) Commission"* the following reference

* I.R. Vol. II, pp. 423 sqq.

is made to attempts to demonstrate changes in the parasite occurring in the mosquito:—

> "I regret that we were on this occasion, as in the case of my previous investigations, unable to demonstrate the evolution of the parasite in the mosquito, though we performed a number of experiments with this object in view. We found considerable difficulties in keeping the mosquitoes alive during the whole duration of the experiments, one of the difficulties being that the guinea-pigs, when exposed to the bites of the mosquitoes, apparently destroyed a considerable number of them. The technique of these experiments had to be worked out very carefully, and it has become evident to me that this important work has but little chance of success when it is carried out, as it was in this as well as in my previous attempts, as one out of several lines of investigation." (pp. 423 and 424.)
>
> "Some observations were however made, which we consider of interest, although they are incomplete; * * * * * *." (p. 424.)

These are no doubt referred to in the summary of the chief results of the work:—

> "III. The demonstration of certain elements—probably division forms of *P. flavigenum*—in infected mosquitoes." (p. 425.)

Epidemiology of Yellow Fever in Relation to *Paraplasma flavigenum*.*

In the following passage Dr. Seidelin discusses the relation of his observations to the established facts regarding the epidemiology of Yellow Fever. It is taken from the earlier paper in the *Journal of Pathology and Bacteriology*.

> "We must now consider the question, how do our observations correspond with the already established facts regarding the epidemiology of yellow fever? In the chief points they evidently agree perfectly by a blood-sucking insect. But there are two circumstances that do not seem to conform with the accepted theory. According to the observations of the American Yellow Fever Commission and others, the microbe ought to have an ultra-microscopical stage in its development, as such is suggested in connection with other diseases, *e.g.*, kála-ázar, instanced by Manson, and this solution would accord with the fact that the microbes are found generally only in small numbers in the blood; it might be that the visible bodies are present in a small minority in comparison with the invisible forms." (p. 287.)

* Journal of Path. and Bact. 15, 1911, p. 287.

"The other circumstances, to which I formerly alluded, is that the germ can only be transmitted during the first three or four days of the disease, whilst the microbes are found for a much longer period. There may be two explanations of this, the first of which is essentially the same as that which has now been suggested, namely, that the ultramicroscopic stage, on which the transmission depends, exists, or is present in the blood, only during the first few days, although the larger forms may remain a much longer period. It ought to be remembered, however, that the observations on which the axiom mentioned has been founded are not very numerous, so that it can as yet scarcely be admitted as an established fact." (p. 287.)

OUR KNOWLEDGE OF THE VIRUS OF YELLOW FEVER.

Before proceeding to consider in detail the various questions which arise in connection with these bodies, it may be of use to set out succinctly the knowledge we possess of the virus of Yellow Fever. We shall thus see more clearly the conditions which must be fulfilled before the organism which Dr. Seidelin has described can be accepted as the active agent in the production of that disease.

(1) It is certainly transmitted by the *Stegomyia fasciata.* It is not known to be transmitted under normal conditions in any other way.

Dr. Seidelin is of opinion that "the axiom that Yellow Fever is transmitted by no other mosquito than the *Stegomyia fasciata*" is "an assertion entirely without proof," and also that "Yellow Fever is transmitted in nature by no other means than mosquitoes has never been proved, but is extremely probable in view of our knowledge of protozoal diseases."

It might have been added "and in view of our knowledge of the effects upon the incidence of the disease afforded by the destruction of the *Stegomyia fasciata.*"

(2) The transmission can only take place after the Yellow Fever parasite has undergone a development in the mosquito, the duration of which is, approximately, twelve days.

Dr. Seidelin considers that "this theory is likely to be correct, but has not been conclusively proved."

(3) The virus will pass through a Berkefeld filter and belongs to the class to which the term ultra-microscopic is usually applied.

It has been objected that the parasite as described by Dr. Seidelin cannot be supposed to be able to pass through a Berkefeld filter.

Dr. Seidelin meets this objection by the suggestion that there may be an earlier stage in the evolution of the parasite in which it is invisible and able to pass through the filter.

(4) The blood of the Yellow Fever patient is infectious only during the early period of the disease, probably not after the third day.

Dr. Seidelin does not accept the view of "the three days' infectiousness"; he believes that "a dogma in medicine has never been established on poorer evidence."

It is not possible within the necessary limits of this report to consider the evidence upon which this view has obtained general acceptance. Those interested in the question who still entertain doubts are referred to the original reports of the American Yellow Fever Commission.

THE ARGUMENT IN FAVOUR OF *P. FLAVIGENUM*.

The argument in favour of the acceptance of these bodies as the causal agent of Yellow Fever appears to involve the admission of the following propositions: —

(*a*) They are found in the blood of patients suffering from Yellow Fever.

(*b*) They are not found in human blood under other conditions.

(*c*) They are found in the blood and tissues of animals (guinea-pigs, white rats, dogs and monkeys) inoculated with blood from Yellow Fever patients.

(*d*) These animals are suffering from Yellow Fever.

(*e*) From such animals by sub-inoculation the disease (Yellow Fever) may be transmitted to successive generations and in the blood the specific bodies are found.

(*f*) If the bodies are found in the blood or tissues of animals which have not been thus dealt with, such animals are the subjects of a natural infection.

(*a*) Presence of the Bodies in Yellow Fever in Man.

In Dr. Seidelin's original paper* five cases are described in which the organisms were found, and fifty cases are referred to.

In the second paper it is stated that† :—

> "In thirty-three cases observed during the last year, after my former article appeared and which were officially declared to be cases of yellow fever, it was found twenty-five times; but of the negative cases six occurred during my absence, and the preparations which I received on my return did not stain well, so they may be excluded. We then get twenty-seven cases with twenty-five positive results. If we include those formerly observed, which formed the basis of the first publication, but which I have not exactly tabulated, we have about eighty cases, in about 90 per cent. of which we have positive findings."

In December, 1911, Dr. Seidelin visited Yucatan on behalf of the Liverpool School of Tropical Medicine to investigate the epidemic outbreak of Yellow Fever which lasted from August 3rd, 1911, to April 30th, 1912. This was the period during which cases occurred which were officially diagnosed as Yellow Fever; the number of these cases was 73, with 38 deaths.

Paraplasma flavigenum was found in the peripheral blood in sixteen confirmed cases out of seventeen in which the blood was examined microscopically. In one of these the diagnosis was made *post-mortem*.

> "The negative was that of J. R. of Kambul. This patient was apyretic and practically convalescent at the time when the blood smears were taken.
>
> "It is not surprising that parasites may be missed at this stage, either because they are absent or extremely scarce. (Y.F.B., Vol. II, No. 2, p. 171.)
>
> "Adding these cases to those of my last two series (1911, 1 and 5) we get 53 positive results in 57 cases. Including, also, my first series, in which the cases were not tabulated, the result is approximately 106 positive cases out of a total of 120." (*Ibid.*, p. 172.)

Paraplasma flavigenum in the Blood in Cases of Yellow Fever at Lagos.

Dr. J. W. Scott Macfie and Dr. J. E. L. Johnston, of the West African Medical Staff, who worked at the Medical Research Institute, Yaba, with Dr. Seidelin, in a paper on "Experiments and

* Berl. Klin. Wochenschr., 1909, No. 18.
† Op. cit., p. 285.

Observations on Yellow Fever,"* state their results with regard to the occurrence of *Paraplasma*-like bodies in the blood in cases of Yellow Fever as follows—

> "We have had the opportunity of examining the blood films from nineteen cases of yellow fever; in sixteen we have detected *Paraplasma*-like bodies in the red blood cells." (p. 125.)
>
> "In our earlier examinations we observed some simple ring-shaped bodies that stained blue. These were present, not only in the red blood corpuscles, but also free in the plasma.
>
> "No chromatin granule could be detected, and although this fact would have been hard to explain were these bodies of a parasitic nature, we considered that the question of their occurrence was worthy of further investigation. Seidelin (1911) has figured bodies presenting very much the same appearance, and has called them 'proto-plasma-bodies without chromatin.' He has suggested that 'the absence of a definite chromatin staining * * * * * * is probably a phenomenon of degeneration.' In one of the first cases examined these bodies were common; but in subsequent cases, although they were occasionally met with, they did not occur with that regularity that one would have expected had they been definitely associated with the cause of the disease. We are therefore inclined to think that they are not of great importance, and although we consider that they may be associated with yellow fever in some manner that is not understood, we do not believe that they are directly concerned in the ætiology of the disease.
>
> "In all those cases of yellow fever from which we have had the opportunity of examining satisfactory blood films, we have detected the presence of minute endo-globular bodies resembling the *Paraplasma flavigenum* of Seidelin; in the accompanying plate (Pl. VI.) a number of these bodies is illustrated. In some cases they were very scarce, but in others they were by no means rare. They were, for instance, more common than malarial parasites are in adult natives suffering from mild attacks of malarial fever, in whom it is often extremely difficult to find conclusive evidence of infection, the parasites being sometimes detected only after examining several blood films, or by employing the thick film method.
>
> "Figures 5-12, Pl. VI., represent different forms of bodies found on several days in a single case of yellow fever in a native. In this case blood films were obtained daily from the first day of the disease until the twelfth day, when convalescence was apparently complete." (pp. 125 to 127.)

Day of the Disease on which the Bodies are present in the Blood

> "It is noteworthy that the bodies were found on eleven out of the twelve days of the illness, and that they persisted after the subsidence of the fever. This fact is not, of course, exceptional for

* Y.F.B. Vol. III, No. 2, pp. 125 sqq.

protozoal diseases of a chronic type, although at first sight it seems remarkable in an acute febrile disorder like yellow fever, which, moreover, is said to be infective only during the first three days. It should be remembered, however, that, as Seidelin has pointed out, this belief rests on a very flimsy basis of fact, and cannot be considered as yet to have received satisfactory scientific proof. It is possible that the persistence of these bodies in the blood during convalescence may explain some of the obscure features of the epidemiology." (p. 127.)

The foregoing statements furnish, in the opinion of the Commission, sufficient proof that these bodies have been found in the blood of a very large number of cases of Yellow Fever; it is, however, hardly necessary to point out that this is only one link in the chain of evidence upon the integrity of which the claim is founded.

If it can be shown that similar bodies are of frequent occurrence in the blood or tissues of man or animals apart from any suspicion of Yellow Fever, it is obvious that the whole contention falls to the ground, and that there is no necessity for any further discussion as to their specific nature.

(*b*) Absence from Human Blood in other Conditions.

Control Observations.

The control observations of Dr. Seidelin and other observers will first be stated and subsequently the cases will be referred to in which Dr. Seidelin himself admits that the diagnosis of Yellow Fever was at least sufficiently doubtful to require careful examination, or to need the construction of a theory to account for the presence of the *Paraplasma flavigenum* under the circumstances in which it was found.*

"In my earlier work I had, quite naturally, examined with special care the blood from yellow fever cases, the control material consisting in the numerous blood examinations occurring in the daily work in my laboratory. This time I proposed to lay special stress on obtaining a large control material. This I did by examining the blood from all cases admitted to the lazaret, from a number of patients seen in the hospital or with physicians in their practice, or from patients whom I had not seen, and, finally, from numerous healthy individuals. In each case I used the same microscope which I used in the yellow fever examinations, always working with the same 3 mm. apochromatic immersion objective (Zeiss) and No. 12 com-

* Y.F.B. Vol. II, No. 2, pp. 176 sqq.

pensating eyepiece; at least one hour was dedicated to the examination of a smear, before it was declared negative. This was carried out in the malarial cases, as well as in those in which no parasites at all were found. The presence of malarial parasites caused but little difficulty. Very young forms, even of *Plasmodium præcox*, were easily recognised by their larger chromatin granule, but occasionally some slight doubt arose when remains of malarial parasites were seen in the erythrocytes, as a rule, after quinine treatment. Such doubt, however, was soon solved by comparison with films containing *Paraplasma*.

"On specially important occasions, special precautions were taken; in such cases blood films were prepared, at the same time, from other individuals and treated all through in the same way as those which were of particular interest.

"In this manner blood specimens were examined on 421 occasions, in each case at least one, and often two or three, slide-smears being thoroughly searched. In 136 cases malarial parasites were found, and in 283 no parasites at all. Finally, in two cases, in which there was no suspicion of yellow fever, *P. flavigenum* was present in small numbers.

"The two individuals, in which *P. flavigenum* was found, were young children seen in a school in Temax on the occasion of a visit which I made with Dr. Casares in order to study the sanitary conditions of that place, from which two yellow fever patients had been brought to the lazaret in Mérida. It was my intention to examine the blood of children with febrile disorders, but we had the opportunity of seeing only two such cases (enteritis), and in both the blood proved negative.

"We then examined the children in the schools, more with the object of discovering cases of malaria, than in the hope of finding cases of larval yellow fever.

"Blood-smears were taken from a number of children with a very slight increase of the spleen dulness (in no case was the spleen considerably increased). Amongst these cases were the two under discussion. As the microscopical examination was made after our return to Mérida the children could not be identified; thus I cannot assert that they were in good health, but, at any rate, they cannot have been severely ill since they went to school. They have certainly not been perfectly healthy, since they were selected for examination as suspicious of malaria. These two cases must be considered together with the two somewhat similar cases which I reported in an earlier paper (1911, 1); in those, *P. flavigenum* was likewise observed in two individuals not suffering from yellow fever.

"The one was a young girl with anæmia, the other a boy with nephritis; both were natives.

"In these four cases we may possibly have to do with microbe carriers, in the true sense of the word—individuals harbouring parasites in their blood for a considerable length of time. But there is no proof that it is so. It may just as well have happened that the blood of

these individuals has been examined during one of the repeated attacks of yellow fever, or, rather, infections with yellow fever parasites, which are supposed by various authors to occur with a certain frequency in natives, producing no characteristic symptoms, or that the individuals have been suffering from chronic infections." (pp. 176 to 178.)

The following occurs in the paper by Dr. Seidelin already referred to*:—

"In these dispensaries" [at Christiansborg and Labadie] "we observed a large number of children, making examinations of the blood whenever we found any rise of temperature or any complaint, the nature of which was not at once clear. Unfortunately the result was negative; in no case was the *Paraplasma flavigenum* found, whilst, on the other hand, malaria parasites were present in the majority of cases.

"An examination of a small number (14) of school-children was likewise negative so far as the *P. flavigenum* was concerned. The only result of a positive nature which we obtained during two months' work" [at Accra] "was the demonstration of *P. flavigenum* in the blood of two patients suffering from mild febrile disorders, not unlike the mild cases of yellow fever, which have been described from various localities by many observers, including myself. The first of these patients was a native of West Africa, but not of Accra; the second case occurred in a European who had arrived in West Africa shortly before he was taken ill." (pp. 421 and 422.)

Doubtful Cases.

"Only in one case was the blood given me before I saw the patient; in this I found the forms seen in Plate XXIX., Figs. 20, 21 and 25, and made the diagnosis of yellow fever, which was at first doubted by the physicians in charge, but afterward fully agreed to. The patient entered the hospital on the third day of his disease, and the preparations were taken the same day.

"In another case, which was diagnosed as typhoid fever, the same corpuscles were observed; but I think that I am able to uphold the clinical diagnosis of yellow fever and will give, with purpose, a short résumé of the clinical history, for which I am indebted to Dr. Vargas, Director of the Lazaret, and to Dr. Vega, to whose service in the hospital the patient was afterwards transferred.

'Case—M.B. aet. 25. Admitted 12 December, 1909. Onset same day with headache and pains in the back. Temp. 103° F. Symptoms increased and the temperature on the third day was 39·7°, descending to 37·2 on the sixth day, rising on the seventh to 38·9; from the eighth day it was about or below 37°. The pulse during the first days was about 100, subsequently it fell to 40 on the eighth day and 38 on the twelfth day. Abdominal pain, at first rather diffuse, was later localised in the epigastrium, especially on pressure. From the third day there was jaundice, first conjunctival, afterwards diffuse. Stools mostly pale, but during several days with melaena. The urine contained albumen, casts, bile pigments and indican; diazo reaction negative.'

* I.R. Vol. II, pp. 412 sqq.

'Widal reaction positive in 1 to 20 1 to 50 and higher negative, so it cannot be considered as conclusively demonstrative of typhoid fever. In the service of the hospital to which the patient was transferred a diagnosis of bilious catarrh was made, but by this time all the febrile symptoms had subsided.'

"This case, therefore, if it be not considered wholly conclusive, can be taken in no wise as demonstrative against the specificity of the microbe; from my point of view, and I followed the clinical course of the malady, it may be looked upon as showing the usefulness of the microscopical examination in difficult cases.

"Another case was observed where influenza was diagnosed, principally because the patient, a negro from Jamaica, was considered immune to yellow fever. Here the corpuscles were also found.

"Serious doubts arose when I found them also in two individuals who certainly did not suffer from yellow fever—in one a young man with a chronic parenchymatous nephritis, and in the other a girl with chronic digestive, somewhat sprue-like, troubles. Both were natives of Yucatan. In the first case there were small forms, in the second only a single free form was found, almost identical with that seen in Plate XXIX., Fig. 39." (Journal of Path. and Bact. 15, 1911, pp. 285 and 286.)

Theories accounting for Doubtful Cases.

"If we consider that the immunity of natives against yellow fever is due, on the consensus of medical opinion, to one or more previous attacks of the disease, atypical and not recognised as such; and, moreover, that the malarial parasites and some piroplasmas, in cattle, for instance, are known to persist for a considerable time without producing any symptoms, then the findings referred to do not seem very much out of line.

"In the case of nephritis, more especially, it would perhaps not be too remote to admit the possibility of yellow fever as an etiological factor, as there was no history of any of the ordinary conditions likely to give rise to nephritis. When I first saw the patient the case looked rather serious, but afterwards it took a surprisingly benign course." (Op. cit., pp. 285 and 286.)

Objections of other Observers.

In Dr. Seidelin's paper* already referred to the following occurs:—

"I would not omit to refer to a paper in which some bodies are described† which show a great similarity to those I have described. These have been seen in quite another disease, namely, *typhus exanthematicus*, and the authors do not discuss the nature of their microbe. If both are confirmed there would be a still further increase in the already numerous protozoal diseases, which in recent years, and more especially in tropical countries, are rapidly acquiring an importance almost as great as that of the bacterial infections."

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* Journal of Anat. & Path. Vol. 15, No. 3, p. 288.

† Krompecher Goldzieher u. Augyán Centralbl. f. Bakteriol. u. Parasit. Jena, 1909, Abth. 1, Orig. Bd. 1. S. 612.

"In my earlier papers* I have already discussed some objections which have been made, mostly in private discussions, against my findings. Since then a few criticisms have appeared, though none of them has been based upon actual observations on yellow fever. The most important is that of Schilling-Torgau (1912—1 and 2), who has found in human blood and in blood from various laboratory animals intracorpuscular bodies very similar to those described by me. The elements which he has observed are, in his opinion, remainders of more or less hypothetical structure which he regards as normal constituents of erythrocytes. This is, of course, a serious objection, being based, as it is, upon careful investigations. The only way to meet it was by a comparative study of blood from other individuals in the same place and with the same technique.

"This is what I have done now, and I think a better material for control than the one discussed above could hardly be obtained. This also answers the objection made at a discussion in Habana by Dr. Guiteras, that I might have encountered an innocent parasite living in the blood of inhabitants of Yucatan."

It may be convenient to state here the reply of Schilling-Torgau to Dr. Seidelin. The article is chiefly of a polemical nature.

"Possibly *P. flavigenum* is the true cause of yellow fever, but its parasitic nature has not yet been determined, much less its etiological relationship to yellow fever, considering the occurrence of very similar bodies in the blood in other conditions."

Archiv. für Schiffs und Tropen-Hygiene (Band. 16, 1912), "Zur Frage des Gelbfiebererregers."

Dr. Agramonte on the *P. flavigenum*.

Dr. Aristides Agramonte, a member of the American Yellow Fever Commission which established the mosquito transference of Yellow Fever, in a paper read before the International Congress of Medicine, London, 1913, "On a Supposed Parasite of Yellow Fever,"† states that he has met with the Seidelin bodies in various conditions which certainly are not Yellow Fever, *e.g.*, goitre, uncinariasis, and Weil's disease. Also in a *post-mortem* blood smear from a case of tuberculosis.

These bodies are also stated to have been present in smears from a guinea-pig which had been treated with human blood.

Dr. Agramonte states that Cartaya has described intracorpuscular bodies in a case of human glanders, and Guiteras in two cases of intense jaundice, not due to Yellow Fever.

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* Y. F. B. Vol. II, No. 2, p. 182.
† Trans. Section 21, Part 2, pp. 71 to 81.

Dr. Agramonte urges various reasons against the acceptance of these bodies as the virus of Yellow Fever, one of which is that in all acute blood infections the parasites usually disappear from the circulation before the last symptoms of the disease, whereas these bodies may be found in Yellow Fever cases up to the eighth or even the fourteenth day.

He considers that "the Seidelin bodies may very well appear in the blood in convalescence from any severe disease, since they represent residual elements produced during a reconstructive process in the blood, and that the blood is often impoverished in Yellow Fever when the attack has been at all marked."

He is disposed "to believe that they are, some of them, chromatoid nuclear fragments of blood elements in the process of reconstruction. That others are protoplasmic residue eosinophilic granules, caused by the hæmolytic action of various pathologic processes."

Dr. Seidelin's Reply.

Dr. Seidelin, in reply, states that having seen Guiteras's specimens from the case of *Icterus gravis*, he has no doubt that they are different from those occurring in Yellow Fever, and that the single indefinite structure which he observed was probably a degenerated malarial parasite. He agrees with Dr. Cartaya that those in the case of glanders were nuclear remains.

Discovery of *P. flavigenum* in Jamaica.

In December, 1912, Dr. Seidelin visited Jamaica at the request of the Liverpool School of Tropical Medicine to investigate the disease called vomiting sickness, prevalent in that island during the winter months and responsible for a considerable mortality, chiefly amongst native children.

In a report of an investigation previously made by another observer the disease had been regarded as Yellow Fever.

As the result of his investigation Dr. Seidelin came to the conclusion that the disease was not Yellow Fever, and that until further evidence may be brought forward we may assume that "vomiting sickness" is a local disease of Jamaica.

In one case, however, in smears of peripheral blood a few intracellular ring-shaped organisms were found of the type of *Paraplasma flavigenum*, and the case is regarded by Dr. Seidelin as having been one of Yellow Fever.

Dr. Agramonte has criticised the diagnosis of this case, and has pointed out the improbability of a single case of Yellow Fever occurring in the course of an epidemic of another disease in an island where Yellow Fever has not been recognised, at any rate officially, for years. This case, if it was one of Yellow Fever, has not been followed by the reappearance of that disease in the long period (now two years) which has since elapsed.

Dr. Seidelin, in reply, maintains the correctness of the diagnosis.*

(*c*) Presence in the Blood and Tissues of Animals Inoculated with Blood from Yellow Fever Patients.

In a paper on Experimental Yellow Fever in Laboratory Animals, by Dr. H. Seidelin and Dr. Andrew Connal,† it is stated that:—

> "In 96 cases" [guinea-pigs injected with blood from cases of yellow fever] "examination of the specimens showed the presence of *P. flavigenum*, whilst 9 cases were negative. In 3 of these, parasites were found in material obtained after death, whilst in 6 cases the results of examination were negative. * * * * * * Thus we had positive results in 99 out of a total of 105 cases examined; about 94·3 per cent."
>
> "In the vast majority of smears the parasite was as scarce as we have usually found it to be in human blood, namely from two or three to about a dozen in a thin film. We found however, in a few cases (as one of us—Seidelin, 1912—has also found in human blood), the parasites in large numbers, comparatively speaking, namely, over fifty in a smear, and sometimes as many as three or four parasites have been seen in one microscopical field. The morphological characters of the parasites were very much the same as those in human blood." (p. 465.)

Presence of Blue Bodies.

> "In these films" [touch preparations from the organs] "ordinary forms of the parasites were found, occasionally in somewhat larger number than they had been found in the peripheral blood, or in the heart blood, but in no case could they be described as very numerous, and often they were quite scanty. Entire absence of the parasites in their ordinary forms was often observed when death took place a considerable time after the inoculation." (p. 466.)

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* Y.F.B. Vol. III, No. 1, p. 69.
† I.R. Vol. II, pp. 464 sqq.

"The most striking elements observed in the organ-touches were extra-corpuscular bodies, which we will call provisionally and for the purpose of description 'blue bodies,' using the term commonly applied to the elements first described as 'Plasmakugeln,' by Koch (1906) and others in East Coast Fever, in cattle. These bodies were in the first instance observed in lung-touches from a guinea-pig which had been killed on the seventh day after inoculation.

"Afterwards they were seen in numerous cases in touch-preparations from the various organs examined, i.e., lung, spleen, bone-marrow and liver, and though as a rule most numerous either in lung or spleen touches, they were very rarely numerous in both these organs in the same animal. The advanced stages of the evolution of the blue bodies were found chiefly in the lungs." (p. 467.)

In the paper entitled "Experiments and Observations on Yellow Fever,"* by Dr. Scott Macfie and Dr. J. E. L. Johnston, from which we have already quoted, the following evidence is given of the presence of *Paraplasma flavigenum* in guinea pigs inoculated with the blood of Yellow Fever patients: —

"Thomas (1907) succeeded in producing a reaction in guinea-pigs from four-and-a-half to thirteen days after being bitten by infected *Stegomyia*, and Seidelin (1912) observed intracorpuscular bodies resembling *P. flavigenum* in two guinea-pigs which he had inoculated from a case of yellow fever. We, therefore, decided to attempt the infection of guinea-pigs by inoculation from the yellow fever cases at Lagos."

"Fourteen guinea-pigs were thus inoculated from eleven cases. A single animal was injected from each of the first ten cases, and four animals from the eleventh. In each case only one or two drops of blood diluted with 1 per cent. sodium citrate solution were used for the injection, which was made subcutaneously. Ten of the animals showed a definite febrile reaction. Three guinea-pigs that were inoculated in a similar manner with normal human blood did not show any such reaction. Positive results have been obtained by inoculations made as late as the eighth day of the disease." (p. 131.)

"Four of the guinea-pigs inoculated failed to show any definite febrile reaction. In two of these, at any rate, auto-erythrophagocytosis was observed. Elsewhere (1913) we have suggested that the phenomenon of auto-erythro-phagocytosis seems to be peculiarly associated with protozoal diseases. Connal (1912), moreover, has suggested that the ingestion of red cells by macrophages may be a natural reaction of the host to combat an infection. In these guinea-pigs, therefore, the occurrence of this phenomenon may have indicated a successful reaction on the part of the host, and may thus explain the absence of fever, the attacks being, in short, aborted. (p. 132.)

* Y.F.B. Vol. III, No. 2, pp 129 sqq.

"That the animals were infected, we believe to be proved by the fact that we detected in the blood of three of them the *Paraplasma*-like bodies that we have found associated with yellow fever.

"In all the guinea-pigs inoculated, with the single exception of the first, No. 49, bodies were found closely resembling the *Paraplasma*-like bodies present in the blood from the human cases. No such bodies were found in healthy guinea-pigs, nor were they present in the blood of the animals before inoculation.

"Seidelin found similar elements in the blood of two of his inoculated guinea-pigs, and stated that 'their resemblance to *P. flavigenum* is also very great.' He considers the question of their being a *Babesia*, such as that described by Baldrey; and also the possibility that they may have been some yellow fever parasites, introduced at the time of inoculation, that had succeeded in surviving in the human blood corpuscles which had been injected. The latter consideration cannot apply in our cases, as the quantity of human blood injected was in every case exceedingly small, and was not introduced directly into the blood stream of the animals. It seems inevitable, therefore, to conclude that these bodies must have been developed in the blood of the guinea-pigs.

"In one case a sub-inoculation was made. One or two drops of blood were obtained from guinea-pig No. 56, which had been inoculated from yellow fever case No. 42, on the sixth day after inoculation, and whilst it still showed some degree of fever, and were injected into guinea-pig No. 63. The latter guinea-pig showed a well-marked, although somewhat irregular, febrile reaction, and on examining its blood *Paraplasma*-like bodies were found to be present." (p. 134.)

Evolution of the Parasite in Animals.

"Some other forms of these bodies encountered in the blood of yellow fever patients are illustrated in Figs. 1-4 and 13-15, Pl. VI. Figures 16-27 represent the bodies found in guinea-pigs, figures 28-39 those found in dogs, and figures 40-44 those found in rats. The earliest form consists of a mere dot of chromatin with a small blue-stained body. In later stages the size of the blue-stained body increases, until a definite ring-form is developed. Several stages of this phase are figured. Ultimately a body of relatively considerable size is produced.

"The general resemblance of these bodies to the *Paraplasma flavigenum* of Seidelin cannot be denied. We have found the same forms, and although we have not been able to make out a definite cycle of development, the types of body illustrated have been constant in our cases. We are aware that Seidelin's bodies have been severely criticised by various authors, but we are, nevertheless, inclined to the belief that they are parasites. We are of opinion that the constancy with which they are found in the blood of yellow fever patients is a matter of considerable importance, and should be a valuable aid to the diagnosis of doubtful cases." (pp. 127 and 128.)

Animal Experiments.

"The experiments on animals, of which an account follows, supply in our opinion, confirmation of the parasitic theory. It would be a remarkable coincidence if bodies of this type were found in human blood from a case of yellow fever, and in the blood of guinea-pigs and dogs inoculated with it, were they not specifically related to the disease." (p. 129.)

Biology of Paraplasma flavigenum.

"As regards the biological nature of these bodies, we agree with Seidelin in regarding them as belonging to the *Babesiidæ*.

"The cycle undergone by the parasite cannot at present be described in detail, several different forms occurring in the blood at the same time, and unfortunately it has been impossible for us to carry out any experiments with infected mosquitoes." (p. 129.)

Paraplasma flavigenum *in Dogs.*

"We have inoculated subcutaneously five dogs with blood obtained from yellow fever patients. Young puppies were chosen for this purpose, as it was thought possible that older dogs might have acquired some degree of immunity to the disease." (p. 135.)

"None of the animals, therefore, showed any febrile reaction, their temperatures remained steady, and they did not appear to suffer any symptoms as a result of the inoculations. Nevertheless, on examining blood films, *Paraplasma*-like bodies were detected in four out of the five puppies. The pathological conditions found at the examination of Dog 96 were probably the result of intercurrent disease, but it is impossible to be certain whether or not they were entirely so.

"We have also examined eleven stray dogs from Lagos that had being procured for us by the police. In the blood of two of these *Babesia*-like, or *Paraplasma*-like bodies were found."* (p. 136.)

"It is, perhaps, too much to suppose that this dog suffered from yellow fever, but in some respects the pathological conditions suggested this disease, and the occurrence of a few *Babesia*-like bodies in the red blood corpuscles, which were very like the *Paraplasma*-bodies found in the dogs inoculated from cases of yellow fever, may be considered to support this hypothesis.

"Our observations on this point have been too few and too incomplete to justify any conclusions. The two dogs referred to certainly appeared to be naturally infected with a *Babesia* resembling the *Paraplasma flavigenum*. It is, of course, possible that the parasite was of another nature; but so far as we are aware, no *Babesia* of dogs has hitherto been described presenting the appearance of that described by us.

* *Footnote by the Authors.* Similar *Babesiæ*, resembling *Paraplasma flavigenum*, have been found by one of us (J.W.S.M.) to be common in sheep and cattle also.

"The experiments on puppies, already referred to, further suggests that it might be possible for dogs to be naturally infected with yellow fever, and, although harbouring the parasite in their blood, to exhibit no symptoms of the disease. In this way they might conceivably become a reservoir of yellow fever, and the disease might be maintained in them." (p. 137.)

REMARKS.

The evidence here given is, we think, sufficient to justify the conclusion that these bodies may be found in the blood of guinea-pigs and other animals which have been inoculated with blood from Yellow Fever patients.

But here, again, the same caution is necessary in accepting the evidence as proof of the specific nature of the parasites, as, if they can be shown to be present in the blood of guinea-pigs and other animals which have not been thus treated, the whole argument fails.

The suggestion that non-inoculated animals in which the parasites are found must be reservoirs of Yellow Fever is ingenious, but not convincing; the more scientific method would be to institute an extensive research in animals of the same species which have never been in a Yellow Fever country.

(*d*) ABSENCE OF THE BODIES IN THE BLOOD OR TISSUES OF ANIMALS WHICH HAVE NOT BEEN INOCULATED WITH BLOOD FROM YELLOW FEVER PATIENTS.

The following extracts from the paper by Dr. Seidelin and Dr. Connal* show that they appreciated the fact that the discovery of these bodies in apparently healthy guinea-pigs, if they cannot be in some way brought into relation with Yellow Fever, is fatal to the argument.

"The question of the natural infection of guinea-pigs by *Paraplasma*-like or other protozoic parasites has received the attention which its great importance requires in our experiments."

The authors naturally assume:—

(1) that the *P. flavigenum* is a parasite;

(2) that it belongs to the order Protozoa; and

* I.R. Vol. II, p. 469.

(3) that if it is found in a guinea pig, or other animal, the animal is suffering from an infection, possibly of Yellow Fever.

"The two safeguards which we adopted with the object of controlling our experiments were the examination of a smear from the peripheral blood of every animal before inoculation, and a complete post-mortem examination of all stock guinea-pigs which died without having been used in the experiments.

"The blood from a total of 170 guinea-pigs and 3 monkeys was examined. All the monkeys showed *Plasmodium*, but none of the stages observed of these parasites resembled in any way the forms of *P. flavigenum*.

"With regard to the guinea-pigs, however, six animals had to be discarded on account of their harbouring in the peripheral blood a minute parasite which resembled *P. flavigenum*.

"These animals were killed, and post-mortem material was taken for examination in the same manner as from the inoculated guinea-pigs. Whilst we consider that the elements which we observed in the blood of these six animals could not with certainty be distinguished from the yellow fever parasite, we can definitely state that in none of these cases were any of the more advanced forms of *P. flavigenum*, which we have described in this paper, found in touch-preparations from the internal organs." (p. 470.)

"It is difficult to explain the mode of infection * * * * * * in the six guinea-pigs which were eliminated from the experiments.

"The infection may have been a chronic or latent one, its inception dating back to some time before their arrival at the Institute. Infection during their transit from their place of origin must also be considered. The possibility of other insect hosts, such as fleas and lice, must be borne in mind." (p. 471.)

In a paper on "Natural Infections in Guinea-Pigs,"* by Dr. A. Connal, Director of the Medical Research Institute at Lagos, and Dr. J. E. L. Johnston, a description is given of the appearances found in 128 guinea-pigs received from Abeokuta and Lagos. Thirty-four guinea-pigs were found to harbour a body believed to be an intra-corpuscular parasite, "although in a few instances the body may have been an artefact."

"The intra-corpuscular inclusion which was regarded as a parasite, so closely resembled the *Paraplasma flavigenum* as to be undistinguishable from it in most cases."

In four guinea-pigs the "blue bodies" previously described in this report were found in the "lung touches." In this paper they are referred to as "division forms."

* I.R. Vol. II, p. 595.

The opinion is expressed in conclusion that "the guinea-pig is very frequently infected in its natural state by a parasite which in its pleo-morphism, but not in its pathological effects, resembles the *Paraplasma flavigenum*." Also that "it is probable that the guinea-pig harbours the true Yellow Fever parasite."

Paraplasma flavigenum IN NORMAL GUINEA-PIGS.

In a Report on the examination of the blood of twenty-five normal guinea-pigs for the presence of Seidelin bodies, undertaken in London at the request of the Commission by Dr. David Thomson,* it is stated that:—

"In all the guinea-pigs except four, bodies resembling Seidelin's Yellow Fever parasites were found."

The blood films were obtained from the ears of the animals.

Sometimes they were very scarce, only one being found after an hour's search. As a rule one fairly definite body containing a red chromatin dot, with a bluish tag or ring attached, could be found within the hour, but other bodies bearing some resemblance to a Seidelin body, were also found. The guinea-pigs examined were obtained in England, and varied in age.

Dr. Thomson states that in some marked blood films sent home by Dr. Seidelin, showing the supposed Yellow Fever parasites in inoculated guinea-pigs, nearly all the bodies shown are more indefinite than he anticipated, and that in his examination of normal guinea-pigs' blood he has seen several such bodies, but has passed them over as artefacts, and as too indefinite to paint.

"The bodies found in normal guinea-pigs bearing a marked resemblance to Seidelin's paintings are depicted in the accompanying coloured plates. In figs. 1-4 signet ring forms are depicted, bluish rings, with a chromatin-like dot on the margin. In some of the signet ring forms the colour of the ring was reddish instead of blue (figs. 11-15). The second group consisted of a chromatin-like dot with a bluish tag of protoplasm attached, as seen in figs. 16-22. Some of these were found free from the red cells, *vide* figs. 23 and 24. The third group consisted of a bluish circle with the chromatin

* I.R. Vol. II, p. 479.

dot in the centre, as in figs. 5-10. The fourth variety consisted of an elongated bluish streak with a chromatin dot in the centre or at one end; some of these were free (figs. 25-28). The fifth group consisted of reddish granules made up of dark and lighter staining parts as depicted in figs. 29-55. The bodies belonging to this latter group were fairly numerous."

Dr. Thomson is of opinion that "with regard to the nature and origin of these bodies no dogmatic statement is possible, but that it is highly probable they arise from various sources. Without doubt the great majority of them are artefacts, and cannot be considered as protozoal in nature, though some of those consisting of a reddish ring with a darker staining centre may possibly be an early stage in the *Lymphocytozoon cobayae* (Kurloff bodies), which were found in practically all of the guinea-pigs examined. The most common origin of the majority of the bodies of the first three groups I consider to be a chance combination of dust, dirt or stain deposit. Stain deposit produces chromatin-like granules, and also, at times, bluish rings of varying size. However clean one may try to make the slide, it is not possible to exclude this source of artefact. In taking blood from a guinea-pig's ear, the film is very liable to be contaminated with epithelial débris, etc., which would take on a bluish stain. Another source of these bodies might be a scratch in the red corpuscle. The leucocyte shown in fig. 38 shows another possible source of chromatin dots, and that in fig. 37 shows another possible source of a complete 'Seidelin body.' A probable organismal source is a type of bacillus, which, when stained with Giemsa, shows a definite blue protoplasm, containing a definite chromatin dot in the centre or at one end, *vide* figs. 26, 27 and 28. This bacillus might explain the origin of group 4. These bacilli are commonly found in the faeces of guinea-pigs, and may easily get on to the blood films from the skin or hair of the animal. I have noticed on occasions that the bodies are sometimes removed from the red cells by cleaning the slide with xylol; this would support the artefact hypothesis."

Lieutenant-Colonel David Harvey, R.A.M.C., in a Report on the results of the examination of normal and inoculated guinea-

pigs,* undertaken at the request of the Commission, states that one hour and a half was given to each slide and as a rule two slides were examined from each animal.

"In practically every slide examined one or two bodies were found which simulated piroplasmata. Some of which are figured are described in detail. These bodies could be divided into the following classes:—

"(*a*) Extraneous, such as bacteria and deposit, sometimes lying between and sometimes on the red cells.

"(*b*) Folds and tears in the red cells, with or without granules.

"(*c*) Polychromatophilia of the red cells with granular stippling.

"(*d*) Occasionally small blue staining rod shaped bodies with a granule, not to be accounted for by (*a*), (*b*) or (*c*), and to all appearance identical with the 'Seidelin bodies.'

"(*e*) Kurloff bodies.

"I laboured under the disadvantage of never having had the opportunity of studying the *Paraplasma flavigenum* under the microscope, but many of the bodies seen and figured resembled the pictures closely."

With regard to the examination of guinea-pigs received from Dr. Seidelin, which had been inoculated with blood from cases of Yellow Fever, and also of those inoculated therefrom, Major Harvey states:—

"Twenty-nine guinea-pigs have been inoculated from these strains; blood films were taken daily for some time and latterly twice a week.

"More than 500 slides were taken, and the majority of them have been stained and examined. Some of the appearances are figured.

"As in the normal guinea-pigs, no definite protozoic parasites of the red cells were seen, although some 'bodies' which simulated such parasites were observed, but rarely, not more commonly than in the normal animals.

"I had the opportunity of examining a stained preparation sent by Dr. Seidelin, showing some stages of a protozoal parasite in the lung of a guinea-pig; in none of the ten guinea-pigs mentioned above was anything resembling this condition seen."

* I.R. Vol. II, p. 731.

(*e*) By the Inoculation of Blood from Yellow Fever Patients that Disease may be produced in certain Animals.

(*f*) From such Animals by Sub-Inoculations Yellow Fever may be Transmitted to Successive Generations and in the Blood the Specific Organisms are found.

The evidence which is relied on to prove the above statements is that:—

(*a*) a certain type of pyrexia is observed in the inoculated animal,

(*b*) albuminuria occurs,

(*c*) the *Paraplasma flavigenum* is found in the peripheral blood during life,

(*d*) the same organism is found in the blood and tissues after death,

(*e*) certain lesions are found in the gastro-intestinal tract and in the kidneys, similar to those observed after death from Yellow Fever.

Remarks.

It is obvious that two questions are here involved, as, apart from any view that may be taken of the specific nature of the "bodies" dealt with in this Report, the proof that the inoculated animals were suffering from Yellow Fever might possibly be accepted on the clinical and pathological evidence alone.

It does not appear to the Commission to be necessary to express any opinion on the general question of the possibility of thus conveying Yellow Fever to animals; all that they propose to consider here is the evidence afforded by the experiments of Dr. Seidelin and others, as detailed in the reports under review, that the particular animals inoculated suffered from Yellow Fever.

Following the order adopted in other sections of the Report, this evidence will now be stated in the observers' own words.*

> "Seidelin (1912) injected guinea-pigs with blood taken from a yellow fever patient on the fifth day of illness and containing *Paraplasma flavigenum*. He found the parasites in the blood of two of the injected animals, but no marked febrile reaction was observed. Another experiment with blood taken from a patient on the seventh day of illness gave a negative result."

* I.R. Vol. II, p. 427. Experimental Yellow Fever in Laboratory Animals. Seidelin & Connal.

"Four guinea-pigs were injected on 4th October, 1913, from the patient No. L 69,* and four others on October 15th from the patient L 73† of the Nigeria cases studied under the Yellow Fever Commission. The former patient * * * * * * was on the fourth day of illness when the experiment was made, the temperature having dropped from 38·9° C. (102° F.) on the previous night, to below the normal. * * * * * * At the time of withdrawing the blood he was on the third day of the disease, and his temperature was about 40° C. (104° F.). * * * * * * Two animals were injected intraperitoneally and two subcutaneously from each patient. The quantity of blood injected varied from 0·35c.c. to 3·5c.c.

"An uninterrupted series of infections was carried on from one of these animals inoculated intraperitoneally from Case L 73. At the end of January, 1914, twenty-three animals had thus been infected consecutively.

"From one of the guinea-pigs in this series another series was carried on.

"Additional animals were infected from time to time, or minor series continued for different periods, for special purposes, or in order to have material to fall back upon should the infection die out in the principal series." (p. 436.)

"The guinea-pigs showed great variations in the way in which they reacted, after infection.

"They showed considerable rises of temperature in some cases, and occasionally marked remissions; in fact, some of the temperature charts might be taken for charts from yellow fever in the human.

"A sudden drop of the temperature occurred in several of these cases, after four to nine days; and in such cases death as a rule took place at this stage, the post-mortem examinations showing alterations typical of yellow fever. More or less marked, but rather irregular, rises of temperature were observed in other cases, and death followed in one to two or three weeks after inoculation, a sudden drop of the temperature just before the fatal issue often occurring also in this group.

"In other cases, again, recovery took place, which was, as far as one could judge, often final, but sometimes only apparent, death supervening one or two months after inoculation.

"Emaciation and albuminuria were the only abnormal conditions found post-mortem in these instances." (p. 428.)

"With regard to the stage of the infection in the guinea-pig from which the blood to be injected was taken, * * * * * * there is no definite relationship between this stage and the severity of the infection produced." (p. 450.)

"We have not found any relationship between the height of the temperature of the animal at the time the blood was withdrawn, and the severity of the infection produced in the sub-inoculated guinea-pig." (p. 451.)

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* *Vide* Dr. Leonard's report. (I.R. Vol. I, p. 268.)
† *Ibid.* (I.R. Vol. I, p. 270.)

" Thus it would appear that the severity of an infection depends more upon other factors than upon the intensity of the infection in the animal from which the inoculations are made." (p. 451.)

" No definite incubation period was apparent. In some cases the temperature rose on the day of inoculation, in others the rise did not set in until two, three or four days after.

" The one symptom of which it is possible in a series of animal experiments to keep a careful record, is the pyrexia.

" The record of the course of the temperature in our cases could not be made as carefully as desirable, for practical reasons." (p. 457.)

" With regard to the normal temperature of guinea-pigs observed under the conditions existing at the place and time of our work, we would say that it shows a considerable range of variation. Our impression is that any temperature between 37·2° and 38·4° C. (99° and 101° F.), may, generally speaking, be considered normal. This, of course, does not exclude that, in individual cases, isolated rises of temperature to or just above 38·4° C. (101° F.) may be pathological, especially if they occur shortly after injection or just before death.

" The temperature, in marked reactions, may rise to or above 39·4° C. (103° F.). * * * * * *."

" The charts, in such cases, show a marked resemblance to those observed in cases of yellow fever in the human, but this condition cannot be laid down as a rule."

" Albuminuria was only observed at the post-mortem examination, as we did not find it practicable to collect the urine from the living guinea-pigs." (p. 459.)

" Omitting the cases in which no urine could be obtained for examination we find that albumen was present in 25 out of 36 cases in which death had occurred spontaneously, and in 9 cases out of 30 in which the animals had been killed. The intensity of the reaction varied from a faint trace, only occasionally observed by Tanret's test, to a dense disc 1 mm. thick, by Heller's reagent.

" It is difficult to make any definite statement with regard to the true mortality due to the infection, as a certain number of non-inoculated guinea-pigs died spontaneously at the same time." (pp. 459 and 460.)

" In addition to these cases of death in the acute stage, we observed several cases in which death occurred from some weeks to two months after inoculation. The post-mortem examination showed an entire absence of lesions corresponding to an acute infection, a more or less marked albuminuria being the only phenomenon noted." (p. 460.)

Anatomical Lesions.

" The most striking lesion observed was undoubtedly a marked affection of the mucous membrane of the stomach, which was present in the vast majority of the cases.

"This affection varied from a more or less marked hyperæmia to an intense acute gastritis with swelling and hyperæmia, hæmorrhages, occasionally in patches, and hæmorrhagic erosions." (p. 460.)

"Other organs appeared but slightly affected. Some cases showed a marked hyperæmia of the lungs, but this was by no means a constant nor even a common phenomenon. Other cases presented moderate hyperæmia of the liver and kidneys, but just as often these organs appeared perfectly normal. The liver appeared distinctly fatty on macroscopical examination, in some few cases. In no instance was any abnormal condition of the spleen recognised macroscopically." (pp. 460 and 461.)

Monkeys.

The observations on three monkeys were incomplete, but after inoculation* *P. flavigenum* was found in the blood of one and "blue bodies" in touch preparations from the lungs. In two other monkeys inoculated parasites were found in the blood smears.

One monkey suffered "a very marked reaction" after inoculation, but its temperature before inoculation had not been observed.

From the curve of the temperature it is concluded that the animal was suffering from Yellow Fever and not from malaria; the parasite of which latter disease was found in its blood. The monkey recovered.

The conclusions of this paper are as follows:—

1. "The yellow fever parasite, *Paraplasma flavigenum* (Seidelin, 1911), has been transmitted to guinea-pigs and monkeys by direct inoculation of blood.

2. "The infection has been carried seriatim through twenty-three guinea-pigs.

3. "The peripheral blood of the infected animals contains forms of the parasite identical with those occurring in human blood in yellow fever.

4. "Various internal organs of the infected animals, particularly the lungs, contain elements which we have found good reason to regard as division forms of *P. flavigenum*" (pp. 475 and 476.)

These division forms are one type of the "blue bodies" to which frequent reference has been made.

These "blue bodies" were demonstrated to the Commission by Dr. Seidelin, and were considered to be identical with the elements described by Carini, and now usually termed "Carini bodies."

* I.R. Vol. II, p. 462.

They have been found in lung smears from guinea-pigs and rats, quite apart from any inoculation with material from cases of Yellow Fever.

From the paper by Dr. Scott Macfie and Dr. Johnston, already referred to, the following extracts are taken: —

* * * * *

"* * * * * * our guinea-pigs, which showed a well-defined febrile reaction following inoculation. The guinea-pigs did not appear to suffer from any other symptoms of disease, but it is often difficult to detect signs of illness in these small animals. Three guinea-pigs, however, died suddenly on the 38th, 20th and 16th day after inoculation respectively. All three had shown a well-marked febrile reaction, but their temperatures had returned to normal some time before their deaths occurred * * * * * * in all three the liver was congested, and the kidneys showed signs of parenchymatous nephritis. In two the urine obtained from the bladder was albuminous.

"As no other deaths occurred among our stock of guinea-pigs at the same time, it would appear probable that the fatal results in these three animals must be attributed to the effects produced in them by the inoculation with yellow fever blood.

"Four of the guinea-pigs inoculated failed to show any definite febrile reaction." (p 132.)

EXAMINATION OF THE EVIDENCE.

It can hardly be claimed that these papers contain a record of the production of a well-defined disease in the animals submitted to experiment. Uniformity in the symptoms could not be expected, for it is contrary to the nature of the acute infectious diseases in man, but before it is accepted that Yellow Fever was produced in these animals, apart from the finding of the bodies named *P. flavigenum*, it would be necessary to prove an assemblage of symptoms and pathological changes presenting a picture which was recognisable, in at any rate a considerable proportion of the infected animals, and strongly suggestive of Yellow Fever.

In the opinion of the Commission the authors have failed to prove either that they succeeded in transmitting Yellow Fever or in producing any well-defined disease.

There is, for example, no suggestion of a period of incubation, followed by a well-marked onset of the symptoms, progressing

through various stages either towards death or recovery, and confirmed in the fatal cases, by pathological lesions unmistakably suggestive of Yellow Fever.

It is recorded that "the guinea-pigs showed great variations in the way in which they reacted after infection": "some cases showed a hyper-acute course, death resulting a few days after the infection," whereas other cases, "although terminating fatally, showed a prolonged course"; others, again, died several weeks after inoculation.

One animal inoculated from a patient in the third day of his illness, when the virus of the disease should have been active, presented no symptoms during the eighteen days it was under observation; whereas another inoculated with blood from a patient on the eighth day of the disease, when the blood should, according to the generally accepted view, have ceased to be infective, suffered from a rise of temperature three days later and continued in a pyrexial condition for eleven days. It is recorded that blood taken on the fifth day and on the eighth day respectively produced acute fever on the day following inoculation, whereas, after inoculation with blood taken on the third day, there was no febrile reaction until four days later. In another case in which the patient was in the second day of the disease "the febrile reaction of the animal was a slight one." Of the second of two similar cases it is recorded that, "as in the previous animal, no febrile reaction occurred." Other animals inoculated from Yellow Fever cases had rises of temperature of which the description is variously given as "well marked," "very slight," "moderate," and "definite, but somewhat irregular."

The following is significant, and tends to throw considerable doubt upon the conclusions arrived at:—

"It is difficult to make any definite statement with regard to the true mortality due to the infection, as a certain number of non-inoculated guinea-pigs died spontaneously at the same time."

One would like to know exactly what number of deaths occurred after inoculation and "spontaneously," and, if possible, from what disease the latter animals died and the lesions found in them *post-mortem*, as such evidence might throw light upon the cause of death in the inoculated animals.

Symptoms.

Pyrexia. One would be disposed to agree with the authors that "the one symptom of which it is possible in a series of animal experiments to keep a careful record is the pyrexia."

Before attempting to draw conclusions as to the effect upon the temperature of inoculating blood from cases of Yellow Fever into any animal, it is obviously necessary to know the ordinary range of temperature in the kind of animal under experiment, and also the effect of inoculation of blood from healthy animals.

Lieutenant-Colonel Harvey's Studies.

With a view to testing this point, the Commission requested Lieutenant-Colonel David Harvey, R.A.M.C., to observe the temperature in twenty-five healthy guinea-pigs in this country, and to compare it with that of guinea-pigs sub-inoculated with blood from animals brought from Lagos by Dr. Seidelin. The latter animals belonged to the series of sub-inoculations referred to in Dr. Seidelin's paper, and were, therefore, supposed to be suffering from Yellow Fever.

He reported as follows:—"The normal temperature of the College guinea-pigs is apparently between 102° and 103° F., and keeps fairly steady. A curious fact is that, on the whole, apart from one or two rises to 104° F., the temperature of the inoculated guinea-pigs was lower than that of the normal."

Having been informed of these observations, Dr. Seidelin, in a "Further Report on Experimental Transmission of *P. flavigenum*," writes as follows:—*

> "Another point which requires further investigation is that of the temperature reactions. Several of the animals showed what appeared to be very marked reactions, but serious doubt as to the importance of these reactions was produced by the statement made by Sir William Leishman that he had observed very high temperatures in non-inoculated animals, and perhaps, on the whole, lower temperatures after inoculation. Since then I have made further observations on this subject, always personally taking the temperatures. I have observed high temperatures also in non-inoculated guinea-pigs, even above 104° F., though the highest temperatures recorded have been in inoculated

* I.R. Vol. II, pp. 485 sqq.

> animals. I am continuing these observations, but, on the whole I should at the present time lay little stress on this symptom. It would appear that the temperatures of guinea-pigs in this country are, on the whole, considerably higher than those observed at Yaba, a point which likewise deserves special investigation. My impression is that the animals often show very high rises after inoculation, and sometimes also very low temperatures, but more especially that they show very considerable variations. It also remains to be seen whether the high temperatures observed in non-inoculated animals are due to insignificant causes, or whether they are due to disease. I have so far been unable to find out that these animals have been suffering from any disease; as mentioned above, no deaths have occurred spontaneously, and the animals appeared quite healthy." (pp. 485 and 486.)

It is obvious from the above that the claim of the authors to have succeeded in conveying Yellow Fever from man to guinea-pigs cannot be based upon the type or degree of pyrexia produced in the inoculated animals.

If this is true with regard to the direct inoculation of blood from cases of Yellow Fever, it appears hardly necessary to examine in detail the evidence on the same point in the cases of the sub-inoculated animals, as the possibility of reproducing the disease by the former experiment is necessarily far greater than by the latter.

Albuminuria.—This is undoubtedly an important symptom in Yellow Fever, but it is not merely the occurrence of albumen in the urine which is significant, but its appearance at a certain period of the disease—at first in small quantity, then gradually increasing according to the severity of the case, persisting in severe cases up to the end, but in milder cases gradually diminishing and finally disappearing altogether.

With the increase in the amount of albumen there is often a diminution in the quantity of urine, varying in degree up to complete suppression. Casts of the urinary tubules are frequently found and may be bile stained. It will be remembered with regard to these animals that in no case was the observation of the urine made during life.

The evidence on this point would have been much stronger if conditions somewhat similar to the above had been observed in the inoculated animals.

The occurrence of albumen in the urine of an animal suffering from pyrexia does not point specially to Yellow Fever, as it is a well-known symptom occasionally accompanying certain febrile diseases in man.

It would also, we think, have been more satisfactory if the tests for albumen used in these experiments had been the common, well-known tests employed in hospitals, as the desire is to bring the observation into line with clinical conditions occurring in man.

There appears to be the same absence of consistency with regard to this symptom as we have already noted with regard to the clinical picture as a whole. For example, one guinea-pig, which had been inoculated with Yellow Fever blood, in which the temperature had been normal for 27 days, died suddenly, and albumen was found in the urine, whereas it should have been absent under such conditions. In another, in which the temperature had been normal for seven days, sudden death occurred, and the urine was found to contain a trace of albumen.

A similar case is recorded in which the temperature had been normal for eight days, when death occurred. The description of the kidneys is as follows:—"The kidneys were congested. In one examined microscopically a number of small hæmorrhages had taken place, the tubules contained a little débris, and their lining epithelium was somewhat swollen." In this case there was no albumen in the urine.

Another guinea-pig, inoculated directly from a Yellow Fever patient, on the fourth day of the disease, showed "a very slight rise of temperature." It was killed on the sixth day. "The kidney was congested, the tubules contained débris, and the lining membrane was swollen and granular." There is no statement as to the condition of the urine.

The clinical value of "a very slight rise of temperature" will be gathered from what has been stated already, yet the kidneys show well marked lesions, although, beyond the discovery of *P. flavigenum*, there is no other evidence that the animal was infected. The question of the value of this evidence as regards both albuminuria and gastric lesions is further discussed on p. 42 *infra*.

Pathological Changes.

The following account is given by Dr. Seidelin and Dr. Connal* of the pathological changes found in the inoculated guinea-pigs:—

> "The most striking lesion observed was undoubtedly a marked affection of the mucous membrane of the stomach, which was present in the vast majority of the cases. This affection varied from a more or less marked hyperæmia to an intense acute gastritis with swelling and hyperæmia, hæmorrhages, occasionally in patches, and hæmorrhagic erosions. Between these two extremes all transitional stages were observed. The hæmorrhages varied in size from minute petechial to confluent ecchymoses of quite considerable dimensions. The erosions were in some cases so minute that it was necessary to use a hand-lens to make certain of their nature; in other cases they exceeded the size of a linseed. No definite localisation of the hæmorrhages and erosions seemed typical; they were observed in all parts of the stomach, though perhaps the cardiac end was somewhat more frequently and more intensely affected than the pyloric and intermediate parts.
>
> "Other organs appeared but slightly affected. Some cases showed a marked hyperæmia of the lungs, but this was by no means a constant nor even a common phenomenon. Other cases presented moderate hyperæmia of the liver and kidneys, but just as often these organs appeared perfectly normal. The liver appeared distinctly fatty on macroscopical examination, in some few cases. In no instance was any abnormal condition of the spleen recognised macroscopically." (pp. 460 and 461.)

Those who have had the greatest experience in the macroscopic and microscopic examination of the organs in Yellow Fever are, we believe, in agreement that in doubtful cases the condition of the liver affords the most trustworthy evidence of the presence of that disease. If therefore the inoculation of these animals had been successful it is, at any rate, highly probable that in a considerable number of them it would have been possible to make a much more convincing statement than that "other cases (it is not stated how many) presented moderate hyperæmia of the liver and kidneys, but just as often these organs appeared perfectly normal. The liver appeared distinctly fatty on macroscopical examination in some few cases."

As with the clinical symptoms, so with the anatomical lesions, the necessary proof depends greatly upon the assemblage of changes commonly met with in Yellow Fever. If, for example, the changes

* I.R. Vol. II, pp. 460 sqq.

observed in the stomach in that disease had been found in association with the typical lesions of the liver and kidneys, and with the presence of albumen in the urine, a strong case would have been made out.

The Condition of the Stomach and of the Urine in healthy Guinea-pigs and in inoculated animals.

With a view to testing the value of the evidence, the Commission requested Major (now Lieutenant-Colonel) D. Harvey to examine the condition of the urine during life in 25 healthy guinea-pigs and in those sub-inoculated from the animals brought from Lagos by Dr. Seidelin, which were supposed to be infected with Yellow Fever.

Lieutenant-Colonel Harvey* states that in the peripheral blood of four guinea-pigs received from West Africa, and in that of 29 normal guinea-pigs inoculated from this strain, bodies resembling the *Paraplasma flavigenum* were found in 15.

Post-mortem Examination.—Twelve of these animals were killed, in ten the mucous membrane of the stomach was normal, whilst in two there were hæmorrhages. In the first eight, which had not been fed in the morning and were killed in the afternoon, the stomach was examined immediately after death, and in all the organ was normal.

As a result of observations on the effect of previous feeding upon the condition of the stomach, it was found that the stomach of an animal inoculated from the West African strain which had been fed showed small petechial hæmorrhages, whereas in another animal under similar conditions, except as regards food taken, the stomach was normal.

An animal inoculated with blood from a normal guinea-pig killed shortly after feeding also showed hæmorrhages and injection of the vessels of the stomach wall.

In the course of observations upon the effect of *post-mortem* changes in producing the lesions of the stomach regarded as characteristic of Yellow Fever, the interesting discovery was made that

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* I.R. Vol. II, p. 740.

if the *post-mortem* examination is delayed for twenty-four hours hæmorrhages appear in the stomach wall and albumen is present in the urine.

Moreover, when the stomach was found to be stippled with what appeared to be small petechial hæmorrhages they all disappeared when the organ was placed in Kaiserling fluid, and on section of the wall no red cells could be seen but only granular débris (hæmoglobin).

Urine.—An important fallacy attending the presence of albumen in the urine of guinea-pigs was discovered, as mentioned above. Thus two guinea-pigs in which during life the urine had been found to be free from albumen were killed at 4 p.m. At the *post-mortem* examination on the following day the urine of both animals was found to be loaded with albumen. One of these animals belonged to the West African series, whilst the other had been inoculated with blood from a normal guinea-pig, yet the result in both was the same, proving clearly that the albuminuria was a *post-mortem* phenomenon and had no relation at all to any supposed infection with the parasite of Yellow Fever.

Touch Preparations.

In two instances "blue bodies" were found in the blood.

In the peripheral blood of three guinea-pigs inoculated from normal animals in exactly the same manner as in the West African series bodies resembling the *P. flavigenum* were found, and in touch preparations of lung and spleen from one of them "blue bodies" were found and were very numerous.

General Conclusions of Lieutenant-Colonel Harvey.

The general conclusions at which Major Harvey arrived as the result of his examination of inoculated guinea-pigs from West Africa and normal guinea-pigs with reference especially to three points, viz.:—

(*a*) The presence of *P. flavigenum.*

(*b*) The significance of petechial hæmorrhages in the gastric mucous membrane; and

(*c*) The occurrence of albuminuria,

were as follows:—

1. Bodies resembling *P. flavigenum* were found in the red cells both of normal and inoculated animals.

2. If the guinea-pigs are killed whilst feeding the vessels of the stomach wall will be found injected, and a few small sub-mucous hæmorrhages may be present.

3. If the *post-mortem* examination is delayed for some hours after death an extreme condition of hæmorrhagic (hæmoglobinuric) stippling may be found in the stomach wall. This colouration disappears in the mounting fluid, and no red cells can be found in section.

4. The examination of the urine of guinea-pigs is liable to fallacy owing to the extreme alkalinity and turbidity of the urine.

Post-mortem, both in normal animals and in animals inoculated from others, albumen is almost invariably present in the urine, and becomes more marked the longer urine is allowed to remain in contact with the bladder.

Report by Dr. C. M. Wenyon and Dr. G. C. Low on the occurrence of *P. flavigenum* in the Blood of Normal Guinea-pigs.

The Commission desire to thank the authors of the above paper (since published in the "Journal of Tropical Medicine and Hygiene," December, 1914, Vol. XVII., No. 24), for permission to incorporate their conclusions in this report.

Their conclusions are:—

"(1) In the blood of normal guinea-pigs, born and bred in England, are to be found bodies indistinguishable from the so-called '*Paraplasma flavigenum*' of Seidelin.

"(2) In most cases these appear to be definite structures, which probably have to do with the development of the red cells.

"(3) They are not parasitic, because they occur in the blood of newly-born animals, not forgetting even the possibility of a placental transmission.

"(4) The apparent success of the inoculation into guinea-pigs of such bodies from Yellow Fever cases is due to a failure of a sufficient examination of control animals.

"(5) The evidence in favour of the Yellow Fever bodies being parasites thus breaks down.

"(6) The presence of such bodies in Yellow Fever bears, therefore, no diagnostic significance, apart from the evidence of blood alteration.

"(7) It is frequently impossible to separate real bodies from pure artefacts, a fact which renders their differentiation one of extreme difficulty."

SUMMARY.

Attention may be directed to the manner in which important facts not fitting in with Dr. Seidelin's theory are met by him. The explanations put forward are not only of a most varied character, but are also often in themselves unsatisfactory. For example:—

In the examination of the blood of certain clinically diagnosed Yellow Fever cases for *P. flavigenum*, "six negative cases," occurring during the absence of the observer, are excluded, because the preparations did not stain well.

A very doubtful case in which the *Paraplasma flavigenum* was found and which was diagnosed as Typhoid Fever and presented a positive Widal reaction in a dilution of 1 to 20, is admitted to be "not wholly conclusive," but "proves the usefulness of microscopical examination in difficult cases."

It is claimed that the clinical diagnosis of influenza in a negro from Jamaica, in whose blood the bodies were found, rested chiefly on the fact that he "was considered immune to Yellow Fever."

When the bodies are found in two patients admittedly not suffering from Yellow Fever, the theory is put forward that they are the remains of previous atypical and unrecognised attacks of that disease, the virus persisting but not producing any symptoms.

As one of the patients was suffering from nephritis, it is suggested that Yellow Fever is a possible etiological factor in nephritis, on the ground that "there was no history of any of the ordinary conditions likely to give rise to nephritis."

The shape of the *Paraplasma flavigenum* is spoken of as though it were uniform in appearance and an easily recognisable object under the microscope; yet fifty figures presenting great variety of form are given. And this notwithstanding that some forms "suggest an affinity to the genus Babesia," whilst others "indicate a relation to the Leishman-Donovan bodies" (*Herpetomonas*), and yet others "present some resemblance to the malarial parasites."

The difficulty as regards the ultra-microscopic character of the virus is met by the suggestion that there are two stages in its development, in one of which it is invisible, whilst in the other it can be seen.

The great difficulty in accepting as the virus of such an acute disease as Yellow Fever, an organism which admittedly is only found in small numbers in the blood, is met by the suggestion that "the visible bodies are present in a small minority in comparison with the invisible forms." That the virus can only be transmitted by the mosquito during the first three or four days of the disease, whereas the "bodies" may be found up to the eighth or fourteenth day or longer, is partly explained by Dr. Seidelin on the assumption that "the ultra-microscopic stage on which the transmission depends exists or is present in the blood only during the first few days," whereas "the larger forms may remain a much longer period."

On reference to the Yellow Fever Bulletin, Volume II., No. 2, Plate II. (facing page 238), one finds: "Figs. 1-15 Forms observed in the case H.S., 1-4 on the forenoon of the first day, 5-8 on the evening of the same day, 9-11 on the second day, and 12-15 on the sixth day." Here it was necessary to prove that an illness of a very doubtful nature was really a second attack of Yellow Fever, and that the parasites were present in the blood during the period in which it is admitted to be infective. It may be mentioned in passing that a second attack of Yellow Fever in a European is not of common occurrence.

Also in the paper on "Experimental Yellow Fever in Laboratory Animals" the "parasites" were frequently found very shortly after inoculation, although previously reported to be absent,* *e.g.*:—

* I.R. Vol. II, p. 464. (Seidelin and Connal.)

"With regard to the first appearance of the parasite in the peripheral circulation, it seems advisable to consider only the small group of twenty cases in which daily examination, of the blood were carried out. One of these cases must be eliminated for reasons presently to be stated. In nineteen cases, then, we found that parasites were present in the peripheral blood; in seven cases after one day, in six after two days, in two after three days, in two after four days, and in one case after six days. In the remaining case, the results were negative during the first eight days of observation, and the blood examination was discontinued thereafter. The case referred to as necessitating elimination is guinea-pig 140: it was injected on 13th October, 1913, and parasites were found in the blood taken before inoculation. It was, however, found afterwards that this animal had been used for another experiment several days previously, in which an intracardial injection of heart blood from an infected guinea-pig had been attempted. This experiment was not considered of any value at the time, as the blood had coagulated in the syringe, but there seems to be no doubt that one or a few drops of blood had actually been injected. Before the first injection, the blood-examinations had been negative." (pp. 464 and 465.)

It will be observed that, as in guinea-pig 140 the blood was found to contain parasites before inoculation, it is suggested that they must have been injected in an experiment which at the time "was not considered of any value." We have already seen, however, that they are frequently found in the blood of normal guinea-pigs.

The following are further examples: —

P. flavigenum was found in twenty-one cases in which Yellow Fever was not diagnosed, but which were all febrile cases of short duration with no local symptoms. One was a case of bronchitis; this leads to the statement that "bronchial affections are occasionally seen in severe cases of Yellow Fever, but in this case I should be inclined to believe that there has been a coincidence of two infections."

The blood of two young children in a school is found to contain the parasites.*

"These two cases must be considered together with the two somewhat similar cases which I reported in an earlier paper (1911, 1); in those, *P. flavigenum* was likewise observed in two individuals not suffering from yellow fever. The one was a young girl with anæmia, the other a boy with nephritis; both were natives.

* Y.F.B. Vol. II, No. 2, p. 177.

"In these four cases we may possibly have to do with microbe carriers, in the true sense of the word—individuals harbouring parasites in their blood for a considerable length of time. But there is no proof that it is so. It may just as well have happened that the blood of these individuals has been examined during one of the repeated attacks of yellow fever, or, rather, infections with yellow fever parasites which are supposed by various authors to occur with a certain frequency in natives, producing no characteristic symptoms, or that the individuals have been suffering from chronic infections." (pp. 177 and 178.)

This involves three propositions none of which are established: —

1. That there are human "carriers" of the virus of Yellow Fever;

2. That this virus consists of parasites, which the carriers harbour in the blood;

3. That chronic infections with the virus of Yellow Fever may occur.

As already mentioned, six guinea-pigs in the Lagos experiments were found to harbour the parasites before inoculation. Thus the authors are compelled to rest their case upon the "more advanced forms of *P. flavigenum*," *i.e.*, the "blue bodies," which were not found in those six animals. But the case for regarding these "blue bodies" as the virus of Yellow Fever is, if possible, weaker than with the other forms. Moreover, there is absolutely no proof that these larger bodies are produced by a process of evolution from the smaller

In discussing the *post-mortem* appearances in thirty-nine stock guinea-pigs which died before inoculation, the authors state*: —

"The remaining two cases require some discussion. The first of these died at an early period of our work, and the blood had not been examined in life. At the post-mortem examination there were hyperæmia and hæmorrhages in the stomach, albuminuria was present, and 'blue bodies' were found in liver and bone-marrow touches. The second case was that of a guinea-pig which had been used for feeding mosquitoes in the room which we had prepared for the breeding of these insects. The blood-examination beforehand had proved to be negative, and at the end of about two months this animal died. The post-mortem revealed the presence of gastric hæmorrhages and of albuminuria, whilst blue bodies were found in the lung, liver, and spleen touch-preparations. Another guinea-pig, whose blood was also free from parasites before being put into the mosquito-house, inhabited the same room and fed the same mosquitoes for the same period of time, and died shortly after the first. The post-mortem findings in this case were negative.

* I.R. Vol. II. pp. 470 sqq. (Scidelin and Connal).

"The parasite found in these cases may have been quite a different one, perhaps another representative of the *Babesiidæ*. But the morphological resemblance, and the presence in the hosts of pathological lesions similar to those observed in the yellow fever infected animals, made us regard it as more likely that the animals had really been infected with *P. flavigenum*. If this is so, the authorities may be right who (Manson and Licéaga) have suggested that endemic yellow fever might be kept up in animals—and the question of prophylaxis would be further complicated. We wish to add, however, that we see no epidemiological reason why this hypothesis should be accepted. Whilst we admit the possibility of this source of infection, we believe that it would in practice prove to be a non-important one.

"It is difficult to explain the mode of infection in the two guinea-pigs whose history has just been described, and also in the six guinea-pigs which were eliminated from the experiments.

"The infection may have been a chronic or latent one, its inception dating back to some time before their arrival at the Institute. Infection during transit from their place of origin must also be considered. The possibility of other insect hosts, such as fleas and lice, must be borne in mind.

"And finally, the question of hereditary transmission by *Stegomyia fasciata* cannot be excluded in the case of the guinea-pig which was used to feed the newly-hatched mosquitoes in the breeding-house, although this theory is not supported by the negative post-mortem results obtained from the other guinea-pig which occupied the room during the same period." (pp. 470 to 472.)

Here again we are asked to accept the following: —

1. That nearly all the lesions relied upon to prove the presence of Yellow Fever in inoculated guinea-pigs may be met with when death occurs "at the end of about two months" after inoculation;

2. Whereas in another guinea-pig which lived under precisely similar conditions and died "shortly after the first" no lesions may be found;

3. That the parasite found in these cases may have been quite a different one, perhaps another representative of the *Babesiidæ;*

4. That it is more likely that the animals had really been infected with *P. flavigenum;*

5. That there may be chronic or latent infection in Yellow Fever;

6. That guinea-pigs may be infected with Yellow Fever whilst in transit in cages from one place to another by the bites of mosquitoes;

7. That there may be insect hosts, *e.g.*, fleas and lice, other than the *Stegomyia;*

8. That the newly-hatched mosquitoes may have been by hereditary transmission carriers of the virus of Yellow Fever and have infected the guinea-pig.

All this is still in the region of speculation.

When the "bodies" cannot be differentiated from those found by others in a disease having no known relation to Yellow Fever, it is suggested that this disease must be of protozoal origin.

> "I would not omit to refer to a paper in which some bodies are described, which show a great similarity to those I have described. These have been found in quite another disease, namely, *typhus exanthematicus*, and the authors do not discuss the nature of their microbe.
>
> "If both are confirmed there would be a still further increase in the already numerous protozoal diseases, which in recent years, and more especially in tropical countries, are rapidly acquiring an importance almost as great as that of the bacterial infections."

Finally, as in one kind of animal after another, viz., normal guinea-pigs, puppies, stray dogs and white rats, the *P. flavigenum* is found, the number of possible reservoirs of the virus of Yellow Fever increases. Dr. Seidelin is, however, not responsible for the following:—

> "The experiments on puppies, already referred to, further suggest that it might be possible for dogs to be naturally infected with yellow fever, and, although harbouring the parasite in their blood, to exhibit no symptoms of the disease. In this way they might conceivably become a reservoir of yellow fever, and the disease might be maintained in them."

In the opinion of the Commission the evidence is conclusive against the claim to have established by the following proofs:—

(*a*) The presence of a protozoon-like body in the blood, the *P. flavigenum*,

(*b*) the occurrence of pathological lesions in the stomach, and

(*c*) the production of albuminuria,

the transference of Yellow Fever to guinea-pigs, either by the injection of blood from cases of that disease, or by inoculation from one infected animal to another.

Conclusions.

The Commission have arrived at the following conclusions upon the subjects dealt with in this Report: —

1. That no proof has been given that the bodies named *Paraplasma flavigenum* are of protozoal nature.

2. That under that name a number of microscopic objects are included.

3. That, excluding artefacts, the origin of most of these objects is at present uncertain, and so far none have been proved to possess any definite physiological or pathological importance.

4. That there is so far no reason to regard any of these objects as the cause of Yellow Fever.

5. That the nature of the virus of Yellow Fever still remains undetermined.

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